

CURRENT-VOLTAGE RELATIONS OF PURKINJE FIBRES IN SODIUM-DEFICIENT SOLUTIONS

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Since Weidmann's (1951) demonstration of a high membrane impedance during the plateau of the Purkinje fibre action potential, it has become clear that the long duration of the cardiac action potential cannot be attributed simply to a 'slowing down' of the processes responsible for repolarization in nerve fibres (Hodgkin & Huxley, 1952), as this would involve a plateau impedance considerably lower than the resting impedance (cf. FitzHugh, 1960). But a maintained depolarization combined with a high membrane impedance could occur if a fall in potassium conductance were to accompany a residual increase in sodium conductance, and suggestions to this effect have already been made (Coraboeuf, Zacouto, Gargouil & Laplaud, 1958; Hoffman & Crane-field, 1960; Brady & Woodbury, 1960).

A decrease in potassium conductance on passing depolarizing (outward) currents across the membrane has been known for some time in the case of skeletal muscle (Katz, 1949; Hodgkin & Horowicz, 1959; Freygang & Adrian, 1961; Adrian & Freygang, 1962*a, b*). The first measurements of the rectifier properties of Purkinje fibres were made by Weidmann (1955), but the presence of a normal sodium concentration did not allow the conductance changes to be assigned to a particular ion species. The present experiments were designed to investigate the voltage dependence of the potassium conductance by measuring current-voltage relations after replacing sodium chloride in the bathing fluid by either choline chloride, sucrose or potassium methylsulphate. Carmeliet (1961*a, b*) and Hutter & Noble (1961) have shown that only a small proportion of the resting membrane conductance of Purkinje fibres is attributable to chloride ions, so that the major part of the resting conductance may be attributed to potassium ions. The fact that the mechanical response of Purkinje fibres is either very weak or absent allows the current-voltage relation to be determined over a large range of potentials, so that the results may be used to reconstruct the changes in potassium conductance which occur during the action potential (Noble, 1960, 1962*a*).

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Some of this work has been reported previously (Hutter & Noble, 1960*b*). Using a similar technique, Carmeliet (1961*a, b*) has obtained results which are in good agreement with those which we have obtained, and Trautwein & Kassebaum (1961) have provided additional confirmatory evidence.

METHODS

Purkinje strands were taken from the hearts of dogs and sheep (Goldenberg & Rothberger, 1936; Draper & Weidmann, 1951). The dogs were anaesthetized with pentobarbitone sodium and the preparations were transferred to warm oxygenated Tyrode solution immediately after excision. The sheep hearts were obtained either from animals which had been used in experiments of a different kind (kindly given to us by Professor Huggett) or from lambs immediately after slaughter at the cattle market. These preparations were immersed in oxygenated Tyrode solution at about 4° C during transport to the laboratory, which usually took about 30 min.

The preparations were mounted in a small channel by means of fine entomological pins inserted through connective tissue or were held down by a piece of nylon stocking. Solutions of the desired composition were forced through the channel at slight pressure from a series of reservoirs at room temperature. Before entering the channel the solutions passed through long polythene tubes lying in a tank of water at 42° C. The temperature drop between this tank and the preparation bath was enough to bring the temperature at the preparation down to 35–37° C. This temperature was recorded by means of a copper-constantan thermocouple.

Membrane potentials were recorded with intracellular micro-electrodes filled with 3M-KCl. The electrode was connected through an Ag-AgCl half-cell incorporating an agar-Ringer bridge to one grid of a push-pull cathode follower input leading to a DC amplifier and oscilloscope. The other grid was connected to a second half-cell and agar-Ringer bridge in direct contact with the bath fluid.

Measurements of membrane resistance were made by inserting a second micro-electrode into the preparation close to the first. Nearly rectangular current pulses were passed through this electrode, the current strength being monitored across a 3 kΩ resistance in the current passing circuit. The current passing electrode was usually filled with M potassium citrate, which passes depolarizing currents more easily than does KCl (Fatt, 1961). Records which showed irregular current pulses were rejected.

The composition of solutions is given in Table 1.

TABLE 1. Composition of solutions (mM)

Solution	NaCl	Choline chloride	Sucrose	KCH ₃ SO ₄	KCl	NaH ₂ PO ₄	NaHCO ₃	Tris buffer (+ acetic acid to pH 7.4)
Tyrode	137	—	—	—	4.0	0.36	12	—
Low Na	—	137	—	—	4.0	0.36	12	—
Sucrose	—	—	274	—	4.0	0.36	12	—
High K	—	—	—	137	4.0	0.36	12	—
Low K, low Na	—	137	—	—	0.4	0.36	12	—
Na-free	—	137	—	—	4.0	—	—	5
Na-free, low K	—	137	—	—	0.4	—	—	5

All solutions also contained 1.8 mM-CaCl₂, 0.5 mM-MgCl₂ and 5.5 mM-glucose. Solutions containing NaHCO₃ were equilibrated with 95% O₂ + 5% CO₂. Solutions containing Tris buffer were equilibrated with 100% O₂.

RESULTS

The effect of substitution of choline for sodium on the membrane potential and membrane resistance

Unlike the sinus and atrium, Purkinje fibres are almost completely insensitive to parasympathomimetic substances (Hoffman & Cranefield, 1960), so that choline may be used as a substitute for sodium. It seemed desirable, however, to verify this point by testing the effect of choline on the membrane potential and resistance. If choline acts as an inert substitute, a small hyperpolarization and, perhaps, a very small increase in resistance would be expected as a result of the removal of sodium ions. Any parasympathomimetic action would lead to a fall in resistance as a result of an increase in potassium permeability. Six experiments were done in which the membrane resistance was measured before and after replacement of sodium by choline. Usually only a very small or no hyperpolarization was seen (cf. Draper & Weidmann, 1951), but in one experiment a hyperpolarization of 15 mV was observed. In no case did any detectable change in membrane resistance occur. Both in Na and choline solutions the resistance to hyperpolarizing currents was less than that to depolarizing currents, and the current-voltage relations were virtually identical up to the threshold for the initiation of an action potential in the Na solution. In sheep Purkinje fibres action potentials were abolished by reducing $[Na]_o$ to 12 mm or less. In dog fibres almost complete removal of Na was required.

Current-voltage relations in choline-Cl solutions

When the current-voltage relation was determined over a large enough voltage range, an S-shaped curve was obtained. The results obtained in sheep Purkinje fibres in low (12 mm) sodium choline chloride solution have been described elsewhere (Hutter & Noble, 1960*b*). The results obtained in a Na-free choline chloride solution are very similar and are shown in Figs. 1 and 2. Figure 1 shows superimposed traces of the electrotonic potentials, depolarization being shown as an upward deflexion from a resting potential of about -80 mV. The calibration (interrupted) lines are at 10 mV intervals. The lower traces show the current pulses, the calibration lines being at 1 μA intervals. Figure 2*A* shows the relation between the polarizing current and the membrane potential at the end of the current pulse (steady-state potential).

It can be seen that the membrane conductance depends on the direction and magnitude of the polarizing current. Thus, a hyperpolarizing current of nearly 2 μA (*b*) produces a voltage deflexion of 35 mV (*B*), whereas a depolarization of 37 mV (*A*) requires only about 0.75 μA (*a*). Neglecting the fact that the current-voltage relation is non-linear, and applying

linear cable theory (Hodgkin & Rushton, 1946), this result means that the membrane resistance to depolarizing currents is 6–7 times larger than that to hyperpolarizing currents for polarizations of this order of magnitude. If the membrane capacity is constant, this difference in membrane resistance should be reflected in a large difference between the time courses of the hyperpolarizing and depolarizing electrotonic potentials. This is in fact the case, and the electrotonic potential rising to a depolarization of

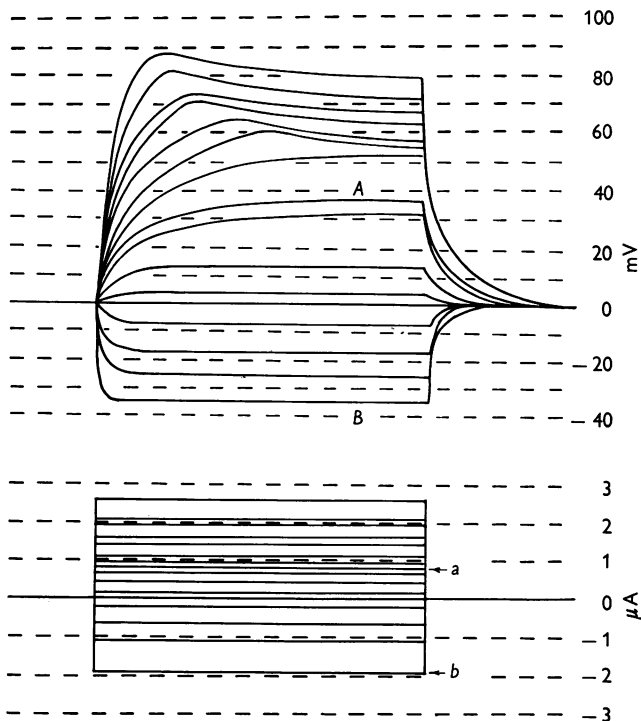


Fig. 1. Electrotonic potentials recorded from a sheep Purkinje fibre in sodium-free choline chloride solution. Above: Superimposed traces of electrotonic potentials. Calibration lines (interrupted) at 10 mV intervals. Depolarization is an upward deflexion from a resting potential of about -80 mV. Below: Superimposed traces of current pulses (700 msec). Calibration lines at μ A intervals. The labelled traces are referred to in the text.

37 mV (*A*) takes very much longer to reach a steady potential than does the corresponding hyperpolarizing electrotonic potential (*B*). For a linear current–voltage relation, the membrane time constant ($r_m c_m$) is given by the time taken for the electrotonic potential at the polarizing electrode to rise to 85 % of its steady-state value (Hodgkin & Rushton, 1946), i.e. in this case to about 30 mV. The time constants determined in this way for these electrotonic potentials are 150 msec for the depo-

larizing potential (*A*) and less than 20 msec for the hyperpolarizing potential (*B*). The estimate of the ratio of membrane resistances obtained from the time courses of the electrotonic potentials is therefore very similar to that obtained by squaring the ratio of steady-state 'effective' resistances. This agreement is encouraging, but too much should not be made out of it because of the non-linearity of the current-voltage relation. One consequence of this non-linearity is that the shapes of the rising and falling phases of the electrotonic potentials are different. Thus, the time constant estimate obtained from the falling phase of the depolarizing electrotonic potential is about 100 msec, which is only 2/3 of the estimate obtained from the rising phase.

On depolarization beyond a voltage deflexion of about 50 mV another phenomenon appears. Instead of rising continuously towards the steady-state value, the potential reaches a maximum, which occurs earlier the larger the depolarization; the potential then declines slowly during the current pulse. The time course of the beginning of the electrotonic potential is still very long. This suggests that on strong depolarization an initial fall in conductance is followed by a slow rise, the speed and magnitude of which depend on the magnitude of the depolarization. Thus, in the case of the smallest depolarization at which this phenomenon appears, the peak depolarization is reached after about 350 msec, whereas in the largest depolarization recorded the peak occurs soon after 100 msec.

The steady-state polarizing current-voltage relation (Fig. 2*A*) is S-shaped, with a maximal slope at a membrane potential of about -40 mV (cf. Carmeliet, 1961*a*). On depolarization beyond about -30 mV the steady-state conductance increases again. This second curvature in the polarizing current-voltage relation occurs at potentials at which the slow increase in conductance is observed during the electrotonic potentials, which suggests that this curvature is a consequence of the time-dependent conductance change.

If the preparation is assumed to be an infinite cable, the steady-state polarizing current-voltage relation may be used to obtain the relation between membrane potential and the membrane current density, i.e. the current-voltage relation which would be obtained if the membrane were to be polarized uniformly instead of at one point. Cole & Curtis (1941) have shown that

$$i_m \propto I \cdot (dI/dV), \quad (1)$$

where i_m = membrane current density, I = total polarizing current and V = voltage deflexion from the resting potential. At various current strengths the reciprocal of the slope of the curve in Fig. 2*A* was measured and multiplied by I to give the relative membrane current density. The resulting curve is shown in Fig. 2*B*. It can be seen that this procedure

exaggerates the curvatures in the relation (cf. Cole & Curtis, 1941; Burke & Ginsborg, 1956), i.e. the cable properties of the fibre have the effect of 'smoothing out' non-linearities in the membrane current-voltage relation. This means that the slow increase in membrane conductance occurring during large depolarizing currents must also be considerably larger than is suggested by the magnitude of the decline in the electrotonic potentials (Fig. 1), particularly as it is likely that only the area of membrane depolarized by more than 50 mV is taking part in the time-dependent changes. Even in the largest depolarizations it is probable that this area of membrane takes a fairly small fraction of the total polarizing current.

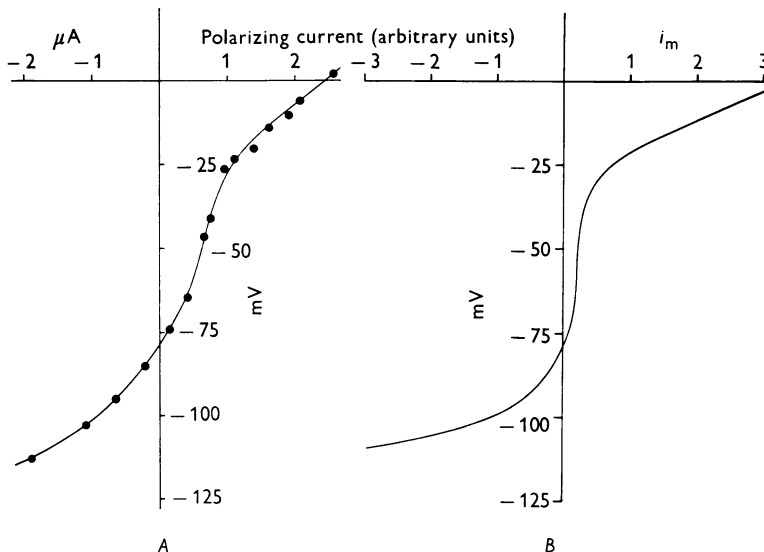


Fig. 2. *A*, relation between total polarizing current (μA) and steady-state potential obtained from records shown in Fig. 1. *B*, relation between membrane current density (in arbitrary units) and membrane potential obtained by applying equation (1) to curve in *A*.

It is likely that the application of equation (1) to these results is not strictly justified, since the fibres were probably only a few space constants in length before terminating or branching. Any error in the calculated membrane current-voltage relation due to this limitation will be most marked over the region of high membrane resistance, since the spatial spread of the potential will then be maximal. It is therefore likely that the fall in membrane conductance on depolarization is overestimated in Fig. 2*B*.

In some preparations the non-linearities in the polarizing current-voltage relation were not so marked as in Fig. 2*A*, and the slow decline in the large electrotonic potentials was either very small or absent. It may be that in these preparations the geometry was least favourable for the detection of resistance changes. Extensive branching would give rise to such a situation. In the limit, when the branching is extensive enough for a 'sheet' membrane to be a closer model of the experimental situation than a 'cable' membrane, even very large resistance changes would be almost undetectable in experiments using point polarization (Noble, 1962*b*).

In experiments on preparations in sucrose solution steady-state polarizing current-voltage relations were obtained which had the same general shape as those in choline-Cl solution. It would therefore seem that the major conductance changes are not due to Cl ions. Carmeliet (1961*a*, *b*) has shown that in sodium-free solution Cl ions are more important as carriers of charge when the membrane is depolarized than in the resting membrane, but he showed that this is most probably due to a decrease in K conductance on depolarization rather than to an increase in Cl conductance. This is in accordance with Hutter & Noble's (1961) conclusion that the anion contributions to the membrane conductance at the resting potential and at the plateau of the action potential are similar. However, more experiments are required to determine the true shape of the anion current-voltage relation.

Current-voltage relations in high [K] solution

The changes in conductance described above may be dependent on the value of the membrane potential or on the direction of the membrane current. This point may be tested by measuring the current-voltage relations when the potassium equilibrium potential is changed, e.g. by depolarizing the membrane in a high [K] solution. It is also of interest to determine the current-voltage relation at K concentrations other than normal, since there are indications in the literature (Weidmann, 1956) that changes in $[K]_o$ may greatly influence the K current flowing during the action potential.

In order to observe the effect of a high [K] solution, the current-voltage relation was first determined in choline-Cl solution, which was then replaced by a solution containing 137 mM- KCH_3SO_4 (high [K] solution). KCH_3SO_4 was chosen in order to avoid the swelling which would eventually occur if potassium were present together with a permeant anion. This also changes the anion composition of the solution, but as g_{Cl} is small this is unlikely to influence the results greatly. During the depolarization following the change to high [K] solution, the membrane conductance was measured at intervals with a small hyperpolarizing current. When the depolarization was complete, a current-voltage relation was determined in the high [K] solution. This is shown in Fig. 3 as the curve connecting the open circles, the curve initially obtained in choline-Cl being shown by the filled circles. The preparation was then allowed to repolarize in choline-Cl solution and another current-voltage relation (triangles in Fig. 3) was measured. A comparison between this and the curve obtained at the beginning of the experiment served to indicate how far the fibre had returned to its original condition. In the hyperpolarizing direction the curves superimpose exactly; in the depolarizing direction the conductance

had increased a little during the course of the experiment. In order to ensure that the fibre had reached a steady state in each solution, about 20 min was allowed after a solution change before measurements were made. The procedure was successfully completed in two experiments which gave similar results.

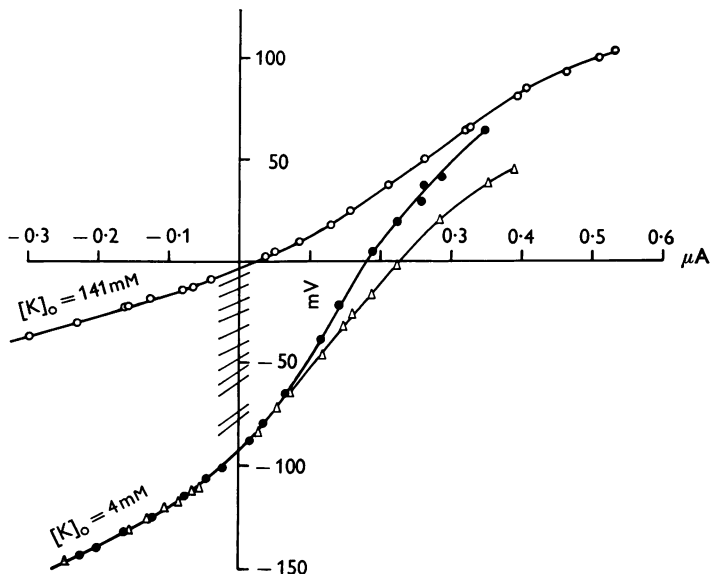


Fig. 3. Steady-state polarizing current-voltage relations obtained successively in choline chloride (low-Na) solution (●), high $[K]$ solution (○), and again in choline chloride solution (△). The lines cutting the voltage axis between the curves indicate the conductances to small currents at intermediate stages during the depolarization.

In Fig. 4 the continuous curves show the membrane current-voltage relation obtained by applying equation (1) to the results in Fig. 3. The mean of the two choline-Cl curves was used. The interrupted curves are relations derived from the constant-field theory by assuming the potassium permeability (P_K) to be constant and to have a value such that the theoretical slope conductance is equal to the experimental value at the resting potential in 4 mM $[K]_o$. These relations are given by

$$I_K = P_K \frac{F^2 E}{RT} \frac{[K]_o - [K]_i \exp(-EF/RT)}{1 - \exp(-EF/RT)}, \quad (2)$$

where I_K = potassium current, E = membrane potential and R , T and F have their usual meanings (Hodgkin & Katz, 1949).

It can be seen from Fig. 4 that in both solutions the deviations from the constant field theory are large: the membrane rectifies in the opposite

direction to that predicted on the basis of a constant P_K . This type of rectification has therefore been called 'anomalous' by some workers (Katz, 1949; Freygang & Adrian, 1961). Carmeliet (1961*a*) has also shown that anomalous rectification is present in high $[K]_o$ in Purkinje fibres.

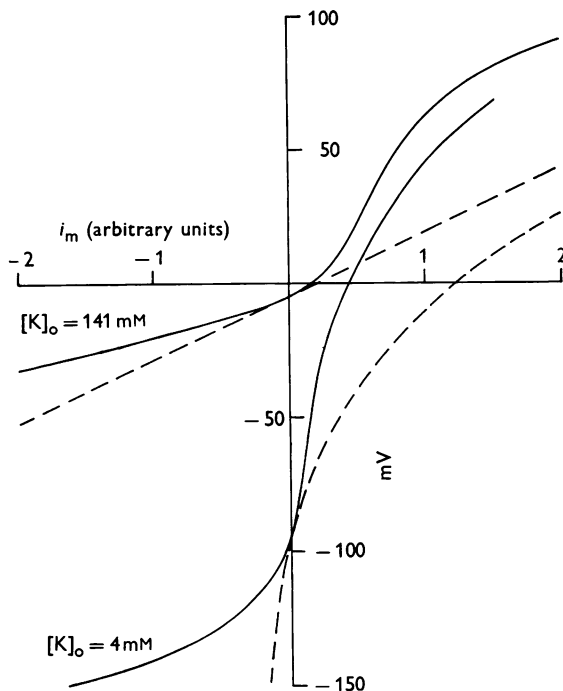


Fig. 4. Continuous curves, membrane current-voltage relations obtained by applying equation (1) to results shown in Fig. 3. Interrupted curves, current-voltage relations obtained from constant-field theory, assuming P_K to be constant (equation (2)). P_K has been chosen so that the theoretical curve in 4 mM $[K]_o$ has the same slope at the resting potential as the experimental curve.

It is interesting to note that the predicted conductance to small currents at high $[K]_o$ is virtually equal to the observed conductance. This result was confirmed in four other experiments and is in agreement with Carmeliet's (1961*a, b*) results. This indicates that, in the absence of large currents, the value of P_K in 4 mM $[K]_o$ is equal to that in 141 mM $[K]_o$.

The relation between the membrane conductance to small currents and the membrane potential at intermediate stages during the depolarization in high $[K]_o$ is plotted in Fig. 5. Since g_{Cl} is small it may be assumed that the membrane potential depends only on the potassium concentration ratio, and as $[K]_i$ is probably constant, the potential depends only on the value of $[K]_o$ in the vicinity of the cell membrane. The conductance-

potential relation predicted by the constant-field theory under these conditions is plotted as an interrupted curve in Fig. 5. The agreement between the experimental and theoretical curves is reasonably good. This lends further support to the view that P_K is constant so long as the potential is close to the potassium equilibrium potential.

The electrotonic potentials recorded in high $[K]_o$ were similar to those in normal $[K]_o$, except that the time constants were smaller as a result of the lower membrane resistance. As in choline-Cl solution, large outward currents produce a small slow increase in conductance during the pulse.

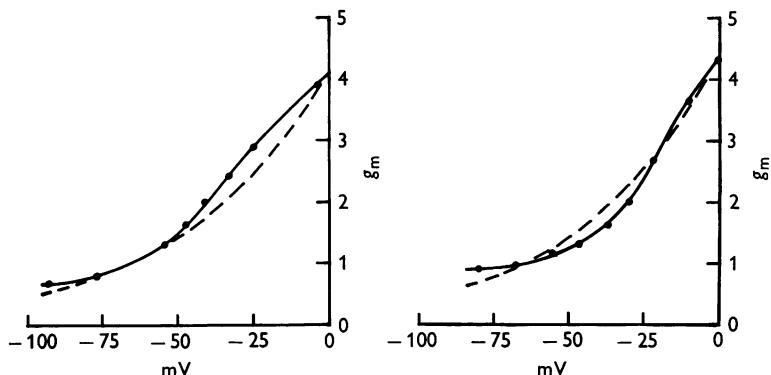


Fig. 5. Relations between membrane potential and conductance to small currents during depolarization in high $[K]$ solution. The interrupted curves are relations derived from the constant-field theory. The continuous curve on the left was obtained from the experiment illustrated in Fig. 3. The continuous curve on the right was obtained from another similar experiment. The units of conductance are arbitrary.

Electrotonic potentials in low $[K]$ solution

The effect of reducing $[K]_o$ to below its normal value depends on the value of $[Na]_o$ (Carmeliet, 1961*a, b*). In a completely sodium-free solution, reduction of $[K]_o$ from 4 mM to 0.4 mM leads to a small hyperpolarization (about 10 mV). In the presence of sodium, even in low concentrations such as 12 mM, the membrane depolarizes in 0.4 mM $[K]_o$ to a potential of about -60 mV. In the latter case there is presumably a very large electrochemical potential gradient tending to move potassium ions outwards across the membrane. It is of interest to record electrotonic potentials under these conditions, since any changes in potassium permeability outlasting the duration of the current pulse should give rise to changes in the membrane potential. In normal conditions the difference between the resting membrane potential of quiescent Purkinje fibres and the potassium equilibrium potential is probably too small for such effects to be observed unless the permeability changes are very large.

The curves on the left in Fig. 6 show superimposed electrotonic potentials obtained in a sheep Purkinje fibre before reducing $[K]_o$ and are similar to those shown in Fig. 1. The figures on the records indicate the current strengths in μA . The potentials recorded in 0.4 mM $[K]_o$ are shown on the right. Several differences are apparent. First, the resting potential fell by almost 30 mV. Since the potassium equilibrium potential will have become more negative, a large electrochemical potential gradient for potassium now exists. Secondly, in 4 mM $[K]_o$ the potentials return to

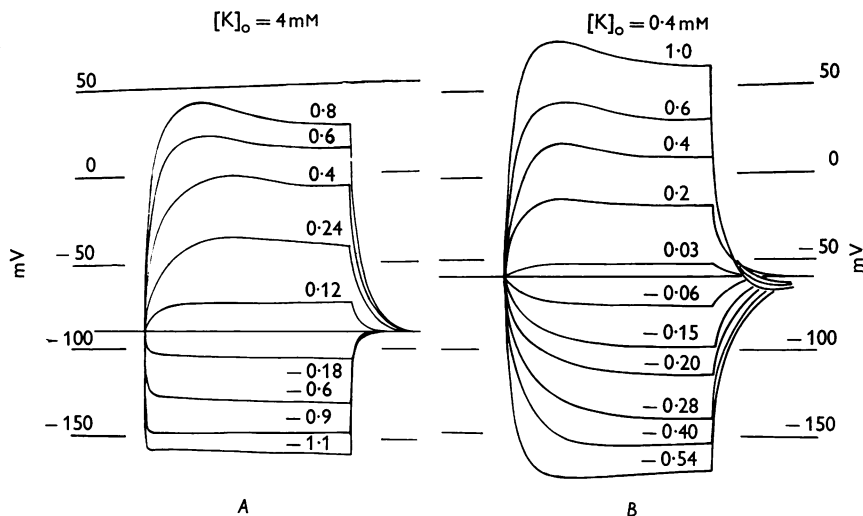


Fig. 6. *A*, electrotonic potentials produced by 700 msec current pulses in choline chloride solution containing 4 mM-K and 12 mM-Na. *B*, electrotonic potentials obtained from same fibre in choline chloride solution containing 0.4 mM-K and 12 mM-Na. Figures on traces indicate total polarizing current in μA . Voltage calibration in 50 mV intervals.

the resting potential at the end of the current pulses, whereas in 0.4 mM $[K]_o$ the potential returns to a more negative value at the end of large depolarizing pulses and then only slowly returns to its previous resting level. The magnitude of this 'undershoot' depends on the magnitude of the preceding depolarization and is greatest when the depolarization is large. This is also the condition for the production of the slow decline in the electrotonic potential during the application of the current, observed in both solutions, and only currents which produce this effect are followed by an undershoot. This strongly suggests that the two effects are due to the same basic phenomenon.

The steady-state total polarizing current-voltage relations obtained from the records in Fig. 6 are shown in Fig. 7*A*. The membrane current-voltage relations are shown in Fig. 7*B*. The main difference between the

curves obtained in the two solutions is that the region of high slope resistance extends to more negative potentials in 0.4 mM $[K]_o$ than in 4 mM $[K]_o$. In 0.4 mM $[K]_o$ the region of maximal slope resistance is near the resting potential and in consequence the time courses of the hyperpolarizing electrotonic potentials are very long compared to those in 4 mM $[K]_o$.

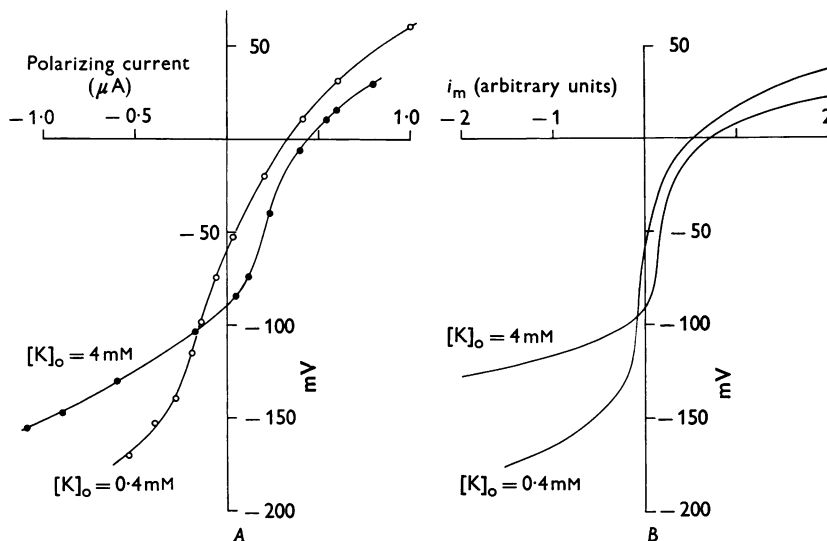


Fig. 7. *A*, relations between total polarizing current and steady-state potential obtained from records shown in Fig. 6. *B*, membrane current density in arbitrary units and membrane potential obtained by applying equation (1) to curves in *A*.

DISCUSSION

The influence of $[K]_o$ on the resting membrane conductance

The results of the experiments on the changes in conductance during depolarization in high $[K]_o$ are in agreement with Carmeliet's (1961*a, b*) results and support the conclusion that the potassium permeability of the membrane (as defined by the constant-field theory) does not depend on the value of $[K]_o$ so long as the electrochemical potential gradient for potassium ions is zero. In addition to electrical experiments similar to those described here, Carmeliet (1961*b*) has also measured the efflux of radioactive potassium at different values of $[K]_o$, and he has shown that at values above 2.7 mM, when the resting potential is probably close to the potassium equilibrium potential (Weidmann, 1956), the increase in rate constant produced by an increase in $[K]_o$ is approximately equal to that predicted by the constant-field theory when P_K is constant.

When $[K]_o$ is reduced below its normal value, the membrane no longer

behaves like a potassium electrode. The membrane potential falls (Figs. 6 and 7) and a large electrochemical potential gradient now tends to drive potassium ions outwards across the membrane. In these conditions the resting potassium conductance falls to a very low value. This observation is also supported by radioactive tracer experiments (Carmeliet, 1961 *b*). The fall in resting K conductance is probably greater than is indicated by the fall in membrane conductance, since the conductance to other ions, such as Cl, which contribute only a small fraction to the normal resting membrane conductance, will become relatively more important when the K conductance is decreased.

When the membrane depolarized in 0.4 mM $[K_o]$ is repolarized by an inward current from an external circuit, the membrane conductance increases again as the potential approaches the probable new value of E_K . In Fig. 7, for instance, the first curvature in the current-voltage relation in 0.4 mM $[K]_o$ occurs at potentials about 50 mV negative to the first curvature in 4 mM $[K]_o$. It is therefore possible that even in 0.4 mM $[K]_o$ P_K may be unchanged when $E_m = E_K$. The evidence at present available is therefore in agreement with the view that the effect of $[K]_o$ on the value of g_K at E_K may be described by the constant field equation

$$(g_K)_{E_K} = P_K \frac{F^3 E_K}{(RT)^2} \frac{[K]_i [K]_o}{[K]_o - [K]_i}. \quad (3)$$

The effect of current flow on the potassium conductance

During current flow large deviations from the constant-field theory occur (Fig. 4). In the case of hyperpolarizing currents, and of depolarizing currents below a certain magnitude, anomalous rectification predominates. An outward electrochemical potential gradient decreases the potassium permeability, whereas an inward gradient increases the permeability. In this respect the Purkinje fibre membrane resembles that of skeletal muscle (Katz, 1949; Hodgkin & Horowicz, 1959; Adrian, 1960; Hutter & Noble, 1960*a*; Freygang & Adrian, 1961). A difference between the two tissues is apparent when the responses to prolonged hyperpolarizing currents are compared. In skeletal muscle a slow fall in conductance is observed (Freygang & Adrian, 1961; Adrian & Freygang, 1962*a, b*). In Purkinje fibres this effect does not occur (see hyperpolarizing electrotonic potentials in Figs. 1 and 6). Anomalous rectification is not, therefore, invariably associated with a slow fall in conductance during hyperpolarization.

When the depolarizing current is strong enough to displace the membrane potential beyond about -30 mV, an effect opposite in direction to anomalous rectification appears, i.e. depolarization produces an increase in conductance. The information which can be obtained with the technique used in our

experiments is not sufficient to describe the time course of this phenomenon in detail but the results indicate that it is similar to the delayed rectification observed in squid nerve (Hodgkin, Huxley & Katz, 1952) in two ways: (1) The conductance increase is greater the larger the depolarization. (2) The conductance increase is faster the larger the depolarization.

There are also two important differences: (3) The magnitude of the increase is much smaller than in squid nerve and it is usually not sufficient to bring the total potassium conductance back to the resting conductance. (4) The speed of the conductance increase is much slower than in squid nerve.

On this view, the effect of a depolarization of the order of magnitude of the action potential will be to produce an initial fall in conductance (anomalous rectification) followed by a rise in conductance (delayed rectification). The experiments in 0.4 mM $[K]_o$ indicate that the slow increase in conductance takes a similar time to decay away again when the membrane is repolarized. This is in agreement with the experiments of Trautwein & Kassebaum (1961), who have shown that a small but significant decrease in conductance occurs during diastole in Purkinje fibres even when the membrane potential is constant.

One relatively simple explanation of the effect of current flow on the potassium conductance is to suppose that potassium ions may move through two types of channel arranged in parallel (Noble, 1960, 1962*a*; cf. Adrian, 1960; Adrian & Freygang, 1962*a, b*), one displaying anomalous rectification, the other showing delayed rectification. In mathematical terms

$$g_K = (g_K)_{EK} F_1(E_m - E_K) + F_2(E_m, t). \quad (4)$$

The function F_1 is less than unity when the argument is positive and greater than unity when negative. An equation for F_1 which fits the part of the membrane current-voltage relation which is uncomplicated by delayed rectification has been given by Noble (1962*a*, eqn. (5)). The function F_2 is clearly similar to the Hodgkin-Huxley (1952) potassium current equations and a modification of these equations which takes account of the differences between delayed rectification in Purkinje fibres and squid nerve has also been given (Noble, 1962*a*, eqn. (6)–(9)). By combining these equations with a slightly modified form of the Hodgkin-Huxley sodium current equations it has been possible (Noble, 1960, 1962*a*) to compute action potentials and pace-maker potentials closely resembling those of Purkinje fibres, and to reproduce the impedance changes described by Weidmann (1951).

SUMMARY

1. The voltage dependence of the potassium conductance in Purkinje fibres of sheep hearts has been investigated by measuring current-voltage relations of the membrane in sodium deficient solutions.

2. In sodium-free choline chloride solution the steady-state current-voltage relation is S-shaped. Hyperpolarizing currents increase the membrane conductance. Depolarizations to about -30 mV decrease the conductance. On further depolarization the conductance increases again.

3. During large depolarizations the electrotonic potentials reach a peak and then slowly decline. The magnitude and speed of this decline both increase as the depolarization increases.

4. In a high K (141 mM) solution the conductance to small currents increases 4-5 times. The current-voltage relation in high K is also S-shaped.

5. In a fibre partially depolarized by reducing $[K]_o$ to 0.4 mM in the presence of 12 mM $[Na]_o$ large depolarizing currents are followed by a transient hyperpolarization. The region of high resistance in the current-voltage relation extends to more negative potentials in 0.4 mM $[K]_o$ than in 4 mM $[K]_o$.

6. The results are interpreted to indicate:

(a) So long as the membrane potential is close to the potassium equilibrium potential the value of the potassium conductance is consistent with a constant potassium permeability as defined by the constant field theory.

(b) Anomalous rectification is present in Purkinje fibres.

(c) During large depolarizations delayed rectification also appears.

(d) A depolarization, similar in amplitude to the action potential, produces an initial fall in potassium conductance followed by a small slow rise.

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